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PROTEIN-CARBOHYDRATE COACERVATES AND β-AMYLASE

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ABSTRACT

The authors study the feasibility of the decomposition of starch in coacervate drops by enzymatic activity to low-molecular products, i.e., sugars. In the solution of the problem use was made of β-amylase, which decomposes starch primarily with the formation of maltose. The enzyme was obtained from soybeans. The preparation was active at pH 3.6 - 7.0. Therefore, for studying its activity, coacervates were selected which form at pH 4.4 - 4.8. In coacervate drops, containing gum arabic, gelatine, starch, and the enzyme β-amylase, the process of decomposition of starch to low-molecular compounds, i.e., sugar, has been shown.

In an earlier report [6] the introduction of α-amylase into a starch-/137* containing coacervate was described, and the activity of this enzyme in the coacervate was established. The action of α-amylase in the drops of the coacervate decomposes starch mainly to dextrins, compounds of comparatively high molecular weight. The present report involves the feasibility of decomposition of starch in coacervate drops by enzymatic activity to low-molecular products - to sugars. For the solution of this problem the enzyme β-amylase was used, which decomposes starch primarily with the formation of maltose. The enzyme was obtained from soybeans [8]. The

* Numbers given in the margin indicate pagination in original foreign text.

preparation was active at pH 3.6 - 7.0. Therefore, for studying its activity, coacervates were selected which form at pH 4.4 - 4.8.

For preparing coacervates, 0.67% aqueous solutions of gelatine and gum arabic were mixed in 5 : 3 ratio. Two ml of the mixture were mixed with 0.25 - 1.0 ml of a 1% aqueous solution of soluble or phosphorylated starch [7, 1]. The volume was brought to 1 ml by addition of distilled water. In one of the experiments 2 ml of soluble starch was added. The obtained mixture was heated 3 min at 40 - 42°C on a water bath and subsequently acidified with 4% acetic acid to pH 4.6 - 4.85, coacervate drops being formed during this process. In all cases, the coacervates and enzymes were studied only after formation of coacervate drops. Their presence in the system was established under the microscope at magnification from 80 to 320 x. On addition of 0.01 N solution of iodine in potassium iodide, the starch-containing drops are dyed to a dark blue-violet color*.

The distribution of starch in the coacervate systems was determined. For this purpose, the test tube with the coacervate was placed in a snow-filled centrifuge beaker and centrifuged 5 min at 3000 rpm. The coacervate drops settled on the bottom and wall of the tube in the form of a precipitate. The coacervates were preserved in drop form in the precipitate. Both precipitate and centrifugate were hydrolyzed with 2% hydrochloric acid. The formed glucose was determined by Bertran's micro-method [2]. The identical coacervate, but without starch, was used in a simultaneous control test (1 ml starch was replaced by 1 ml water; the replacement was required to obtain the same volume and ratio of components). Subsequently, from the amount of glucose obtained by hydrolysis of the precipitate and centrifugate of the starch-containing /138
coacervate, the amount of glucose was calculated which was obtained by hydrolysis of precipitate and centrifugate from a coacervate which does not contain starch. The amount of starch was calculated by multiplying the determined glucose by 0.9. The percentage of starch in the precipitate and centrifugate was calculated in relation to the total amount of starch in the total coacervate (see Table 1).

*If coacervate drops are prepared from gelatine and gum arabic and the blue-violet 1% solution of soluble starch with iodine is added to the drops, the drops slowly adsorb the solution, becoming colored while the surrounding liquid turns colorless. This phenomenon is similar to the absorption of dyes by coacervates [5], but the absorption of the high molecular iodine-starch compound is interesting.

TABLE 1

Distribution of starch in coacervates

Starch in the mixture in ml	Starch content in mg: in precipitate	in mg: in centrifugate	in mg: in total coacervate	in %: in precipitate	in %: in centrifugate	in mg per ml: in precipitate	in mg per ml: in centrifugate
Soluble starch							
0.5 (3.3 mg dry material)	0.178	2.39	2.57	6.93	93.00	1.78	0.82
0.5 (3.83 mg dry material)	0.334	2.216	2.55	13.10	86.90	3.43	0.76
2.0	0.371	10.42	10.79	3.44	96.56	-	-
Phosphorylated starch							
0.4	0.11	1.86	1.97	5.58	94.42	1.10	0.64

The data of Table 1 indicate that: 1) The coacervate drops in precipitates contain starch. 2) At constant composition of other components of the coacervate, the percentage of starch bonded in coacervate drops varies with the amount of starch added. 3) At a starch content of 10.77 mg and more in the total coacervate the percentage of starch decreases in the fraction corresponding to the coacervate drops. The same effect is shown at starch contents of 2.57 mg or less. 4) The highest percentage of starch is contained in the coacervate drops if the total coacervate contains 2.55 mg soluble starch or 1.97 mg phosphorylated starch. 5) The absolute amount of soluble starch in coacervate drops at total starch contents of 2.55 mg and 10.79 mg is highly similar (0.334 and 0.371 mg). 6) The total volume of coacervate is 3 ml, that of precipitate after centrifugation is 0.1 ml, and the amount of starch calculated per unit volume indicates that in the coacervate drops, forming the precipitate, the concentration of starch is 2 and even 4.5 times higher than in the surrounding solution (see Table 1). The entry of starch into the coacervate drops was not further studied, but the data obtained permit the assumption of a saturation limit of coacervate drops with starch, which can not be changed by a further increase in the amount of starch. Obviously, the starch is bonded by the protein-carbohydrate component of the coacervate.

In preparing such coacervates, the purity of the reagents and especially of the soluble and phosphorylated starch is very important.

Unfortunately, the starch content of a single coacervate drop could not be determined in the present study. Large differences, however, can hardly be expected between the starch concentration in all coacervate drops and in each of these drops. This assumption appears highly probable, considering studies of the amount of ribonucleic acid in all coacervate drops and in an individual coacervate drop [3]. These studies gave similar magnitudes. Deviations from the average values are possible, of course. /139

Determination of the Enzymatic Activity of β-Amylase in Coacervate.

In order to determine the enzymatic activity, solutions preheated to 42°C were mixed, using 2 ml of a mixture of 0.67% solutions of gelatine and gum arabic (5 : 3), 0.5 ml of 1% soluble starch, and 0.5 ml of 0.05% solution of β-amylase. The mixture was acidified to pH 4.82 - 4.85 with acetic acid. The coacervate formed was heated at the same temperature for 15 min on the water bath. Subsequently, the mixture was centrifuged under cooling as described. The reducing power of sugar, formed by reaction of β-amylase with starch, was calculated in ml of $KMnO_4$. Control tests with β-amylase inactivated by boiling were simultaneously carried out. The experimental results are presented in Table 2.

TABLE 2

Activity of β-amylase with starch in coacervates

Starch	Variation	0.01 N $KMnO_4$ in ml			
		precipitate		centrifugate	
		1	2	1	2
Soluble (0.5 ml in mixture)	Test	0.65	0.75	3.45	3.70
	Control	0.19	0.20	0.20	0.20
Phosphorylated (1.0 ml in mixture)	Test	1.10	1.24	7.46	7.42
	Control	0.62	0.57	0.33	0.33

The data of Table 2 indicate that β-amylase decomposes starch both in the precipitate (in the phase of coacervate drops) and in the centrifugate. The total volume of coacervate solution is 3.0 ml, the precipitate representing 0.1 ml. For 2.9 ml centrifugate, 3.25 - 3.50 ml of 0.01 N $KMnO_4$ solution are used in titration (subtracting the blind test), thus, (approximately) 0.11 - 0.12 ml should be used for 0.1 ml, and the test with the precipitate showed that 0.45 - 0.55 ml of 0.01 N $KMnO_4$ is consumed. Therefore, the concentration of sugars in coacervate drops (precipitate) is approximately 4 times higher than in the surrounding drops of solution (centrifugate). It is possible, however, that maltose penetrated into the coacervate drop or that it was adsorbed onto it from

the surrounding solution. To study this possibility, 0.2 ml (1.69 mg) of maltose solution was added to the coacervate, containing β-amylase inactivated by boiling. The coacervate with maltose was stored for 15 min at 42°C and after centrifuging under cooling the maltose in the precipitate and centrifugate was determined from its reducing power, reported in ml of $KMnO_4$, Control tests without maltose were run simultaneously. The test results are given in Table 3.

TABLE 3

Determination of maltose in coacervates

	0.01 ml N $KMnO_4$ in ml	
Coacervate	centrifugate	precipitate
Maltose	2.90	0.31
Without maltose	0.22	0.31

The data of Table 3 show that maltose is present only in the centrifugate, whereas the coacervate drops (precipitate) do not contain maltose. Therefore, the presence of maltose in coacervate drops is due to the reaction of β-amylase with starch present in these drops.

To obtain additional data supporting the presence of amylase in coacervate drops two coacervates were prepared: I - from gum arabic, /140
gelatine, and soluble starch, and II - from gum arabic, gelatine, soluble starch, and β-amylase. Subsequently the coacervates were centrifuged and to the precipitate of coacervate II, containing ferment, we added the centrifugate of coacervate I, which contained soluble starch, and vice versa. The mixture obtained was heated and stored for 15 min at 42°C, centrifuged, and the reducing power in ml $KMnO_4$ was determined (see Table 4). Control tests after inactivation of the enzyme by boiling were run simultaneously.

TABLE 4

Determination of β-amylase in coacervate

	0.01 N $KMnO_4$ in ml			
	Test		Control	
Fraction	precipitate	centrifugate	precipitate	centrifugate
Precipitate of coacervate II + centrifugate of coacervate I	0.94	2.83	0.13	0.16
Precipitate of coacervate I + centrifugate of coacervate II	0.14	2.91	0.16	0.14

The data of Table 4 show that β-amylase is present in the tercomponent coacervative (containing gum arabic, gelatine, and starch solution), in coacervate drops (precipitate), and also in the residual liquid (centrifugate). The enzyme preserves its activity both in coacervate drops and in the surrounding liquid.

Conditions are known where amylase, adsorbed on a precipitate (with high water content), hydrolyzes starch [4]; thus, the activity of β-amylase, contained in coacervate drops, is not unexpected.

In the present study, the coacervate drops were formed from the turbid precipitate. Possibly, in remote times in the bodies of water on the Earth, the emergence of organic compounds preceded a phase of formation of precipitates from organic compounds. Organic materials were concentrated in the precipitates and the latter were a good material for formation of coacervate drops. In the coacervate drops the concentration of organic compounds was also much higher than in the surrounding aqueous solution.

In coacervate drops, containing gum arabic, gelatine, starch, and the enzyme β-amylase, the process of decomposition of starch to low-molecular compounds, i.e.,sugar, has been shown.

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